Understanding the Role of the Circadian Clock Gene, Bmal1, in Astrocytes

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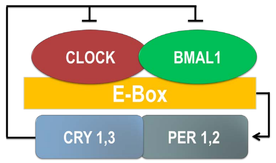
Introduction

The endogenous circadian rhythms in mammals are driven by a network of clock genes. These clock genes in turn regulate behavioral and physiological changes that follow a rhythm of approximately 24 hours [1]. The functions of all of the different types of brain cells in this process are not completely known and characterized. It is known that the brain region, the suprachiasmatic nucleus (SCN) in the hypothalamus, is essential to maintain the body's circadian rhythm. The role of the neuronal cells in this circadian rhythm has been studied extensively. However, recent studies have shown a circadian rhythmicity in glial cells, such as the astrocytes, as well [2]. Even without complete characterization of glial cells, there are reasons to consider astrocytes as crucial to circadian rhythm: they secrete gliotransmitters such as ATP in a circadian fashion, express circadian levels of glial fibrillary acidic protein (GFAP), and the disruption of glial metabolism has shown to alter circadian locomotor activity in some experiments [3].

In view of these recent findings, we propose to investigate the role of the principal glial cells (astrocytes) in the overall circadian rhythmicity in mice. Among the core network of clock genes, Bmal1 is a key gene which when ablated induces arrhythmicity at molecular and behavioral levels.



This study is intended to advance the current knowledge in the circadian rhythm field. It will add to the growing body of knowledge on the role of glial cells in circadian rhythm beyond the original idea that the central circadian pacemaker of mammals is solely driven by neurons. Understanding the role of the glial cells in circadian rhythmicity in mice, may help understand the same in humans. That in turn may provide insight into and help treat diseases that are correlated with chronic disruption of the circadian clock; such as diabetes, some cardiovascular diseases, and cancer [4].

Research Question

We hypothesize that the lack of Bmal1 in astrocytes will impact circadian rhythmicity, at the molecular level (gene expression via assessment of core clock gene Per2 expression), as well as in terms of whole animal behavior (running wheel activity on different lighting regimens).

Materials and Methods

Astrocyte Specific BMAL1 KO: An astrocyte specific BMAL1 KO mouse line was created through breeding Tg(GFAP-*Cre*) and *Bmal1*$^{flx/flx}$ mice. GFAP-Cre transgenic mice from founder line 73.12 have Cre recombinase expression directed by the mouse glial fibrillary acidic protein promoter. Cre expression is observed in astrocytes in the brain and spinal cord, as well as postnatal and adult GFAP-expressing neural stem cells and their progeny in the brain.

Immunohistochemistry and Imaging: 100 micron coronal brain sections were created from both WT (control) and GFAP-BMAL1-cKO mouse brains and were examined by co-staining for BMAL1 and one of the following: glial fibrillary acidic protein (GFAP) or neuronal nuclei (NeuN). The primary antibody, which included rabbit anti-Bmal1 (1:200) and one of the following: mouse anti-GFAP (1:200) and mouse anti-NeuN (1:200), was applied for 3 days at 4C. The secondary antibody containing Alexa Fluor 488 goat-anti-rabbit (1:200) and Alexa Fluor 594 goat-anti-mouse (1:200) was applied overnight (4C). The success of the GFAP-BMAL1-cKO mouse was assayed via confocal microscopy.

Actogram: The ability for the control and KO mice to maintain circadian rhythms was assayed by first entraining to a 12 hours of light and 12 hours of dark (12:12 LD) daily schedule. After approximately two weeks of 12:12 LD entrainment, the mice were placed in 24 hour darkness (DD) for 3 weeks. Lastly, the mice were placed in a skeleton light protocol which consists of a 1 hour, 1000 lux light pulse every 12 hours. Each cage included a monitored running wheel, which recorded every turn of the wheel, creating waking/sleeping pattern data which was then analyzed to show any effects on circadian rhythmicity [5].

Bioluminescence: Photomultiplier tubes experiments were done with astrocyte cultures by placing control and KO samples (GFAP-*Cre* and *Bmal1*$^{flx/flx}$ bred with Per2 to create a triple knockout) in a Lumicycle to assess gene expression as shown below. The Lumicycle measures every time a photon is released.

Astrocyte Culture **Lumicycle (Actimetrics)**

BMAL1 Selectively Knocked Out in Astrocytes

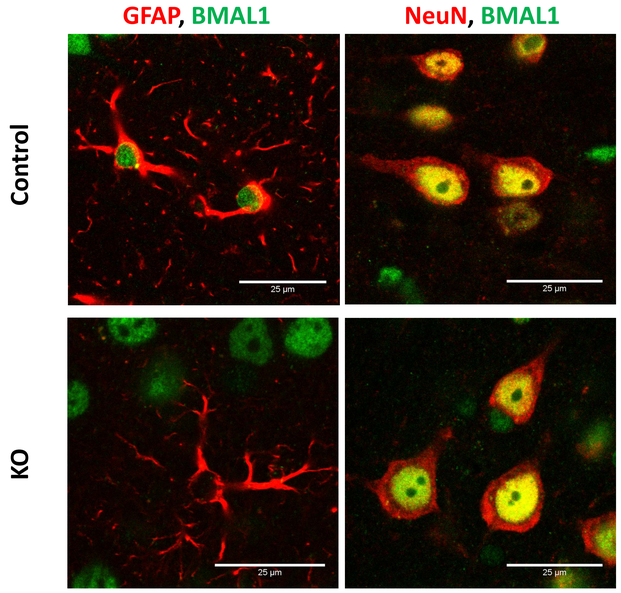


Figure 1. Bmal1 in neurons and astrocytes in control and knock out mice. Green indicates Bmal1 and red indicates the cell type. As shown, Bmal1 was successfully knocked out of astrocytes (GFAP), but remained in other cell types (NeuN).

mPER2::LUC Astrocyte Culture

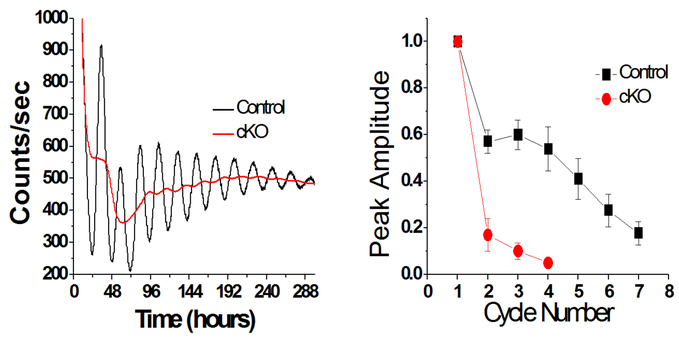


Figure 2. Rhythmicity of the mPER2::LUC astrocyte culture *in vitro*. Dramatically decreased rhythm seen in KO. (Marker for clock not functioning).

BMAL1-cKO Mice Maintain Locomotor Circadian Rhythmicity

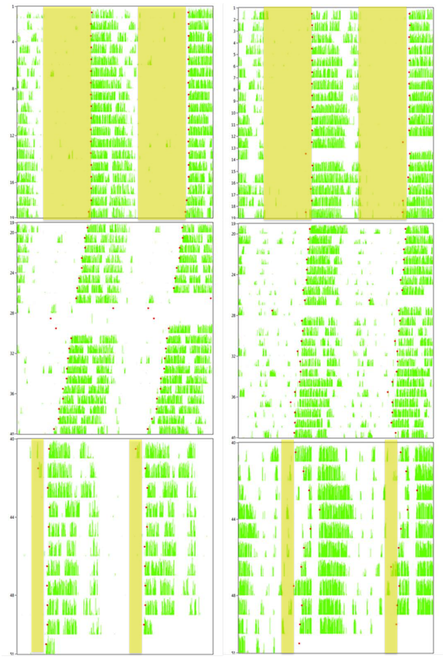


Figure 3. Circadian rhythmicity is not significantly different between WT and GFAP-BMAL1-cKO mice. From top to bottom A and B show running wheel data from WT and GFAP-BMAL1-cKO mice ages ranging from 8-26 weeks, respectively. Green represents active running wheel use. After a few days of 12:12 LD conditions acclimation, data was collected for 2 weeks LD (top panel), 3 weeks DD (middle panel), and 2 weeks of skeleton lighting protocol (bottom panel). There was no significant difference between each group in terms of amplitude or period in any of the lighting regimens. Averages shown below.

	Control		KO	
	period	amplitude	period	amplitude
LD	24.01	7979.44	24.00	12072.20
DD	23.66	7515.00	23.85	12704.05
SKEL	23.82	6982.23	24.20	6194.39

KO n=6, WT n=5

Summary and Conclusions

- *In vitro* control astrocyte cultures maintain a rhythmic expression of Per2, while knocking out BMAL1 causes arrhythmic expression of Per2.
- Running wheel data indicates no difference in locomotor activity between control and knock out mice suggesting the gene in astrocytes is not essential to maintain circadian rhythmicity.
  - PMT clock knockout of BMAL1 does not always lead to locomotor problems.

Future Directions

- Same experiment plan with new mouse line (B6.Cg-Tg(Gfap-cre)77.6Mvs/2J) which should have no Cre recombinase activity in postnatal or adult neural stem cells (or their progeny) as well as in astrocytes to better selectively target astrocytes.
- Investigate any evidence of astrogliosis in these mouse lines with the use of imaging of hippocampal regions and Western blots to quantify GFAP expression.

References and Acknowledgements

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